Intramolecular Cleavage of LexA and Phage λ Repressors: Dependence of Kinetics on Repressor Concentration, pH, Temperature, and Solvent†

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ABSTRACT: LexA repressor of *Escherichia coli* and phage λ repressor are inactivated in vivo and in vitro by specific cleavage of an Ala–Gly peptide bond in reactions requiring RecA protein. At mildly alkaline pH, the in vitro cleavage reaction also proceeds spontaneously, suggesting that peptide bond hydrolysis is an activity of the repressors rather than of RecA. The spontaneous cleavage reaction, termed "autodigestion", has been characterized for the LexA and λ repressors. The results show that the reaction is intramolecular. The rate of LexA autodigestion was studied over the pH range 7.15–11.77 and over the temperature range 4–46 °C. The logarithm of the rate constant increased linearly with pH and reached a plateau value (2.5×10^{-3} s^{-1} at 37 °C) at pH above 10. The data closely followed a model in which a single residue side chain (apparent pK = 9.8 at 37 °C) must be deprotonated for the protein to show activity. Analysis of the temperature dependence gave the heat of proton dissociation as 19.9 kcal/mol and the heat of activation for hydrolysis as 15.3 kcal/mol at 25 °C. Autodigestion of λ repressor, studied over the pH range 8.65–10.70 at 37 °C, was similar to the LexA reaction in its pH dependence, yielding a pK of 9.8. The maximum rate at 37 °C for λ repressor, 6.1×10^{-5} s^{-1}, was 40 times slower than for LexA, a difference similar to that previously observed in vivo and in vitro for RecA-dependent cleavage reactions. There was no significant solvent deuterium isotope effect on the autodigestion of LexA. Changes in buffer composition, including high concentrations of glycine for λ repressor and of imidazole or hydroxylamine for LexA, indicated that solvent components other than water do not participate in the rate-determining step. Removal or addition of metal ions did not significantly affect LexA autodigestion. These and other observations suggest that the deprotonated form of an amino acid side chain plays a central role in the chemistry of the cleavage reaction. The above observations establish repressor autodigestion as a member of an emerging set of biologically important self-processing reactions.

The SOS regulatory system of *Escherichia coli* is controlled by the action of two proteins: the LexA repressor, which regulates a set of about 20 genes during normal growth, and the RecA protein, which is required for inactivation of the LexA repressor after treatments that damage DNA or inhibit DNA replication [reviewed by Little and Mount (1982) and Walker (1984)]. LexA repressor is inactivated by specific cleavage of an Ala–Gly bond near the center of the polypeptide chain (Little et al., 1980, 1981; Horii et al., 1981a). Treatments that induce the SOS response also result in the induction of temperate phages such as λ, P22, and 434 (Roberts & Devoret, 1983; Johnson et al., 1981). Phage repressor inactivation similarly is mediated by cleavage at an Ala–Gly bond near the center of the chain (Phizicky & Roberts, 1980; Craig & Roberts, 1980; Sauer et al., 1982a; DeAnda et al., 1983; Anderson et al., 1984).

These repressors are small proteins (LexA, 22 300 Da; λ repressor, 26 200 Da)[1] with known amino acid and DNA sequences (Sauer, 1978; Markham et al., 1981; Horii et al., 1981b; Sauer et al., 1982b). They consist of an amino-terminal domain that binds specifically to operator DNA and a carboxy-terminal domain that mediates dimerization. The two domains are connected by a protease-sensitive hinge region containing the Ala–Gly cleavage site (Pabo et al., 1979; Sauer et al., 1979; Johnson et al., 1980, 1981; Little & Mount, 1982; Little & Hill, 1985). In addition to the conserved Ala–Gly bond, there is considerable homology in the carboxy-terminal domain (Sauer et al., 1982b).

Genetic and biochemical studies have shown that an activated form of RecA protein is required for cleavage in vivo and in vitro (Little & Mount, 1982; Little, 1983; Roberts & Devoret, 1983). The in vitro cleavage reaction requires a ternary complex of RecA protein with two cofactors—single-stranded DNA and a nucleoside triphosphate—which are considered to be the activating components in vivo as well (Phizicky & Roberts, 1980; Craig & Roberts, 1980). Until recently, the simplest interpretation of these findings was that activated RecA protein is a specific protease.

However, this picture has been called into question by our finding that cleavage of both LexA and phage λ repressors can take place at mildly elevated pH in the absence of RecA protein (Little, 1984). This reaction, which we term autodigestion, is similar to the RecA-dependent reaction in its specificity. Both reactions cut LexA protein at the same Ala–Gly bond, and both reactions are strongly inhibited by the *lexA3* (Ind⁻) mutation (Little & Hill, 1985), which changes the Ala–Gly cleavage site to Ala–Asp (Markham et

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[1] Abbrevations: Da, dalton; bp, base pair; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; DTT, dithiothreitol; CAPS, 3-(cyclohexylamino)propanesulfonic acid; HPLC, high-pressure liquid chromatograph; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; IPTG, isopropyl thiogalactoside; kb, kilobase.

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al., 1981). Moreover, cleavage of λ repressor is slower than that of LexA in vivo and in the RecA-dependent and autodigestion reactions in vitro. These properties suggest that the two types of cleavage reaction proceed, at least in part, by a common reaction pathway and that RecA protein acts indirectly to stimulate the autodigestion reaction rather than directly as a protease. Understanding the mechanism of autodigestion, therefore, bears on the goal of understanding the overall mechanism of repressor cleavage.

In order to pursue these questions further, we have studied the kinetics of autodigestion of the LexA and λ repressors. These studies confirm our earlier tentative conclusion that autodigestion is an intramolecular reaction. We also report the dependence of the reaction rate on pH, temperature, and solvent composition. The data suggest that a protein group with a pK near 10 facilitates the hydrolysis reaction and that no solvent species other than water participates in the rate-limiting step.

Materials and Methods

Plasmids and Bacterial Strains. Strains of *E. coli* K-12, with only relevant genotypes listed, were as follows: AB1157 *lexA*$^+$ *recA*$^+$ (Bachmann, 1972); JL467 = DM511 *lexA41* (Ts)/F′*lacI*^{q} (Little & Hill, 1985); JL468 = AB1157/F′*lacI*^{q} (Little et al., 1981); JL652 = JL468/pJWL59; JL915 = JL468/pSNS101; JL905 = JL468/pSNS103. Plasmids pJWL59, pSNS101, and pSNS103 are described below; pJWL42 was described by Markham et al. (1981).

Enzymes. Restriction enzymes, BAL31 nuclease, DNA polymerase large fragment, and T4 DNA ligase were obtained from New England Biolabs. Restriction enzymes were used as directed by the supplier; BAL31 was used according to Stroynowski et al. (1982); DNA polymerase large fragment and T4 DNA ligase were used according to Maniatis et al. (1982).

Plasmid Construction. The construction of pJWL59, which contains the *lexA* gene under the control of the *tac* promoter (De Boer et al., 1983; Amann et al., 1983), is described in Figure 1. The wild-type phage λ *c*I gene was transferred from the control of the *lac*UV5 promoter, as present on the plasmid pKB280 (Backman et al., 1976; Backman & Ptashne, 1978), to the *tac* promoter by excising an 830-bp *Msp*I–*Taq*I fragment of DNA from pKB280 and inserting it into the unique *Cla*I site of the plasmid pEA300 (Amann et al., 1983; a similar scheme with a 1100-bp *Hpa*II fragment was employed by these workers). This plasmid was designated pSNS101. The *ind*^{s}-1 mutant of phage λ repressor, hereafter called *ind*^{s}, was also placed under the control of the *tac* promoter by use of a similar construction scheme, except that the source of the 830-bp *ind*^{s} fragment was the plasmid pBK2 (Cohen et al., 1981) and the vector was pSH1, a shortened version of pEA300 in which the sequences between the *Hin*dIII site and the *Pvu*II site (approximately 2000 bp including the inactive tetracycline resistance gene) had been removed by ligating the *Pst*I–*Hin*dIII fragment from pEA300 with the origin-containing *Hin*dIII–*Pst*I fragment from pJWL59. Preliminary characterization of this Ind^s overproducing plasmid (pSNS103) revealed a 20–30% greater overproduction than with pSNS101. This may be due to a higher plasmid copy number resulting from the removal of the *rom* gene (Tomizawa & Som, 1984).

Repressor Proteins. LexA protein was purified from strain JL652 as described previously (Little, 1984). λ Repressor and its Ind^s mutant form were purified from strains JL915 and JL905, respectively, by use of a modification of the method described by Johnson et al. (1980). Cells grown in YT broth (0.8% bactotryptone, 0.5% yeast extract, 0.5% NaCl) and induced with 1 mM isopropyl thiogalactoside for 90 min were pelleted, resuspended in L buffer (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, 5% glycerol) at 0.1 g of cell paste/mL, and treated with lysozyme (1 mg/mL) for 30 min on ice. Cell lysis was accomplished by adding 1 M potassium phosphate (pH 6.8) to a final concentration of 400 mM. Following centrifugation (Beckman SW27 rotor at 83000g and 4 °C for 60 min) to remove cellular debris and high molecular weight DNA, the supernatant was treated with poly(ethylenimine)-HCl, pH 7.9 (0.6 %) for removal of remaining nucleic acids. Repressor protein in the supernatant was precipitated with ammonium sulfate (0.4 g/mL), resuspended in L buffer without NaCl, and applied to a hydroxyapatite column. After washing, the column was developed with a linear gradient from 0.1 to 1.0 M potassium phosphate (pH 6.8) containing 2 mM β-mercaptoethanol. Repressor eluted between 0.55 and 0.75 M phosphate. By SDS–PAGE (Laemmli, 1970), the protein of this fraction appeared to be greater than 98% pure. The repressor fraction was dialyzed against 10 mM Tris-HCl, pH 7.5, 200 mM NaCl, 1 mM DTT, and 1 mM EDTA and was stored at 4 or −70 °C.

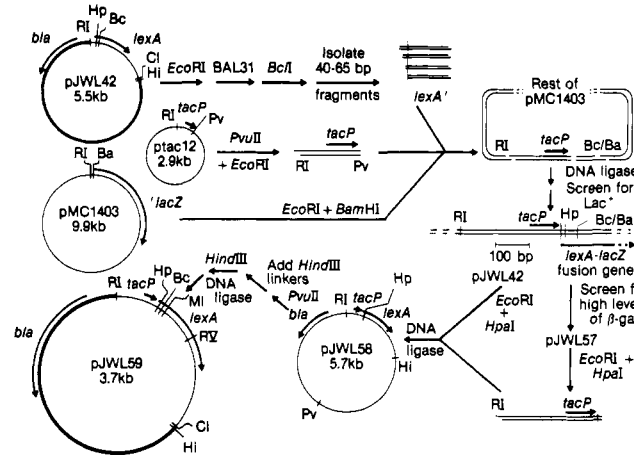

FIGURE 1: Construction of the plasmid pJWL59. First, a tripartite fusion [cf. Guarente et al. (1980)] between the *tac* promoter, the start of *lexA*, and most of *lacZ* was made from p*tac*12 (Amann et al., 1983), plasmid pMC1403 (Casadaban et al., 1980) cut with *Bam*HI at amino acid 8 of *lacZ*, and plasmid pJWL42, carrying *lexA*. pJWL42 was cut with *Eco*RI, treated with BAL31 followed by DNA polymerase I, dCTP, dGTP, dTTP, and [α-^{32}P]dATP (Maniatis et al., 1982), cut with *Bcl*I, and run on a 12% polyacrylamide gel; fragments of 40–65 bp were eluted from the gel and ligated with the *tac* promoter and *lacZ* fragments. Following transformation of JL468, Lac$^+$ clones were identified by their blue reaction on X-Gal plates and assayed for β-galactosidase production (Miller, 1972) in the presence of the inducer isopropyl thiogalactoside (IPTG). Second, the fusion of *tac* promoter to the start of *lexA* was subcloned from pJWL57, the clone making the largest amount of enzyme, into pJWL42 to reconstitute *lexA* with a fusion to the *tac* promoter. Following transformation of JL467 and selection for LexA function (Little, 1980), a colony sensitive to IPTG was isolated and shown to overproduce *lexA* protein by treatment with IPTG, followed by SDS gel electrophoresis (not shown). Finally, the plasmid from this clone, pJWL58, was reduced in size by about 2 kb as shown, by use of a synthetic linker, 5′-P-d-(CCAAGCTTGG) (Collaborative Research). This step removed several cloning sites present in the *tet* gene and part of the plasmid *rom* gene, which is involved in controlling copy number (Tomizawa & Som, 1984). Following induction with IPTG, this strain made 10–20% of the cell protein as LexA protein (data not shown).

Production of [^{35}S]*Methionine-Labeled Repressors.* Strains JL652, JL915 and JL905 were grown in M9 glucose medium (Miller, 1972), supplemented with a mixture of seven amino acids (Little, 1983), to a cell density of about 5 × 10^8 cells/mL. Two minutes following induction with 1 mM isopropyl thiogalactoside, [^{35}S]methionine (New England Nuclear Corp.;

ca. 1000 Ci/mmol) was added to a final concentration of 50 μCi/mL, and the cells were allowed to grow for an additional 5-min period (Little, 1983). Repressor proteins were purified as described above for λ repressor and its Ind^s mutant and for LexA as described previously (Little, 1984), except that the methylmercury and hydroxyapatite columns were omitted. Under these conditions, purified repressors were estimated to have a specific radioactivity of about 1×10^6 cpm/μg.

Proteolytic Fragments of λ Repressor. Prior to use, papain (Boehringer-Mannheim; 10 mg/mL suspension) was diluted to 50 μg/mL and activated by incubation at room temperature for 10 min in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 5 mM cysteine. A mixture of purified radiolabeled and unlabeled λ repressor (93 μg/mL with a specific activity of about 4×10^3 cpm/μg) was treated with activated papain (12.5 μg/mL) at ambient temperature for 25 min in buffer A (10 mM Tris-HCl, pH 8.0, 200 mM NaCl, and 1 mM EDTA) with 2 mM $CaCl_2$ and 5 mM cysteine. At the end of the treatment period, papain activity was inhibited by the addition of $ZnSO_4$ to a final concentration of 10 mM and the digestion mixture was separated on a QAE-Sephadex column equilibrated with buffer A containing 10 mM $ZnSO_4$. Repressor fragment d, which comprises the amino-terminal domain (Pabo et al., 1979; Sauer et al., 1982a), flowed through, and fragments a, b, and c were eluted from the column with 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 10 mM $ZnSO_4$, and 1.0 M NaCl. This fraction was diluted 5-fold in the final reaction mixture and analyzed for fragment a autodigestion activity as described below.

Buffers and pH Measurements. The pH was determined with an Orion meter (Model 501) equipped with a combination glass electrode (Orion 91-03) and calibrated with standard buffers (pH 7.0 and 10.0 at 25 °C) at the measurement temperature, taking into account the effect of temperature on pH of the standard buffers. The pH of autodigestion reactions was measured at the reaction temperature. Unless specified otherwise, the buffer for autodigestion reaction consisted of equimolar amounts (50 mM) of Trizma base (Sigma), glycine (Sigma), and 3-(cyclohexylamino)propanesulfonic acid (CAPS) (Calbiochem), called TGC buffer. The pH was adjusted by adding HCl or NaOH to 2-fold concentrated stock solutions. TGC buffer gave a linear dependence of pH on added acid or base between pH 7.8 and pH 11.6 at 25 °C.

Assay of the Autodigestion Reaction. Reaction mixtures typically contained radiolabeled repressor protein in a buffer containing 200 mM NaCl, 10 mM $CaCl_2$, 0.01% bovine serum albumin (Bethesda Research Laboratories), and 50 mM TGC buffer, adjusted to the desired pH. At selected times, aliquots of the reaction solution were mixed with an equal volume of 125 mM Tris-HCl, pH 6.8, 4% SDS, 10% β-mercaptoethanol, 20% glycerol, and 0.05% bromphenol blue to stop the reaction. The cleavage products were separated by SDS–PAGE (13%) (Laemmli, 1970). Samples of low protein concentration were mixed prior to electrophoretic analysis with partially autodigested, unlabeled repressor as a marker. When sample volume was too large for direct loading onto gels, protein was precipitated with 5% trichloroacetic acid and 0.1% deoxycholate. The gels were stained by soaking overnight in a solution containing 25% isopropyl alcohol, 10% acetic acid, and 0.025% Coomassie Brilliant Blue (Fairbanks et al., 1971). Following destaining in 7% acetic acid, the three protein bands, representing uncleaved repressor and carboxy-terminal and amino-terminal fragments, were excised, placed in scintillation vials containing 0.5 mL of 90% NCS tissue solubilizer (Amersham), and incubated at 42 °C for 2–3 h. Glacial acetic acid (15 μL) was added to each vial, followed by 5 mL of ACS scintillation fluid (Amersham). Radioactivity was determined by scintillation counting, and the extent of repressor cleavage for each sample was calculated as the ratio of the total number of counts in the two cleavage bands to the total radioactivity in all three bands. Recovery of label was greater than 90%. Triplicate experiments, used for most of the rate determinations, yielded values showing less than 10% uncertainty.

Fitting of Data. Data were fit to kinetic models by use of the simplex algorithm of Nelder and Mead (1965) with a modification of their quadratic method for extimating variance. Estimated uncertainties given for parameters are standard deviations. A copy of the program, coded in C or Basic, will be supplied on request.

Amino Acid Sequence Determination. A 135-μg sample of λ repressor in 500 μL of 10 mM Tris-HCl, pH 7.5, 400 mM NaCl, 1 mM EDTA, and 1 mM DTT was mixed with an equal volume of 200 mM bicarbonate buffer, pH 10.53, at 25 °C and incubated at 37 °C for 48 h. Analysis by SDS–PAGE showed that cleavage had gone to completion. To 500 μL of the cleaved sample, trichloroacetic acid was added to a final concentration of 5%. After 30 min on ice, the precipitate was collected by centrifugation at 16000*g* for 10 min and the pellet was resuspended in 100 μL of water. This sample was mixed with 3 mg of Polybrene and subjected to 5 cycles of automated Edman degradation in a Beckman Model 890M sequenator. Detection of phenylthiohydantoin derivatives was by use of a Beckman Model 334 HPLC.

Removal of Divalent Metal Ions. Approximately 500 μg of purified LexA protein was dialyzed against 100 mL of 100 mM Pipes, pH 7.0, 6 M guanidine hydrochloride (Bethesda Research Laboratories), 200 mM NaCl, 10 mM EDTA, 10 mM EGTA, and 10 mL of settled Chelex (Bio-Rad). Prior to use, the Chelex was washed with 2 volumes of 0.5 M sodium acetate, pH 6.3, followed by 10 volumes of deionized water, on a Büchner funnel lined with Whatman No. 1 filter paper. The denatured LexA protein was renatured by dialysis against two 100-mL changes of the same buffer, except the concentration of Pipes was reduced to 10 mM and guanidine hydrochloride was omitted. A final dialysis was performed against 10 mM Pipes, pH 7.0, 200 mM NaCl, 1 mM EDTA, and 1 mM EGTA. Water and all buffers were treated with an equal volume of settled Chelex prior to use.

Solvent Isotope Effect. The effect of D_2O on LexA autodigestion was performed in the pH range 8.65–10.70 at 37 °C as described above. For each pH, two aqueous autodigestion buffers, containing all constituents except LexA protein, were lyophilized and reconstituted, one with H_2O and the other with D_2O (Alfa; 100% deuterium oxide). Similarly, two samples of an aqueous LexA preparation (approximately 4×10^3 cpm/μg) were lyophilized and reconstituted with H_2O or D_2O. An aliquot of the LexA protein solution was added to an autodigestion buffer reconstituted with the same solvent, and the rate of autodigestion was determined. The pD was determined as pH meter reading + 0.4 unit (Jencks, 1969). The concentration of D_2O in reaction mixtures was greater than 97%.

Approximately half of the LexA protein was cleaved during lyophilization. This observation is of interest in connection with changes in reaction rate and protein properties at low hydration levels (Rupley et al., 1983). Cleavage rates determined for the H_2O/D_2O comparison are for the material remaining intact, which broke down with first-order kinetics.

RESULTS

Comparison of the Bond Cleaved through Autodigestion

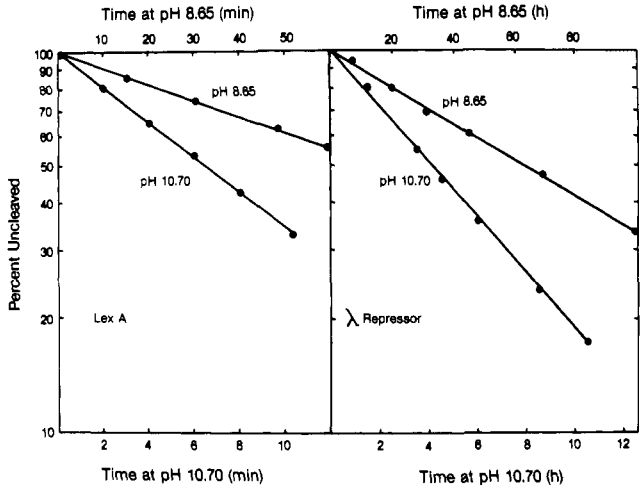

FIGURE 2: Time dependence of LexA and λ repressor autodigestion at pH 8.65 (upper scale) and 10.70 (lower scale). Reactions were carried out at 37 °C as described under Materials and Methods with a total repressor concentration, expressed as concentration of monomers, of 6×10^{-6} M for LexA protein and 1×10^{-9} M for λ repressor. Each data point represents the average of three experiments and has an uncertainty of less than 10%.

and RecA-Dependent Cleavage of λ Repressor. It has been shown that autodigestion of LexA protein cleaves the same Ala–Gly bond that is cut in RecA-dependent reactions (Little, 1984). We found this to be true for λ repressor as well. Amino acid sequence analysis of the autodigestion products of λ repressor gave an approximately equimolar mixture of Ser-Thr-Lys-Lys-Lys and Gly-Met-Phe-Ser-Pro. These sequences correspond, respectively, to the amino acid sequences of the amino- and carboxy-terminal fragments of λ repressor following RecA-mediated cleavage (Sauer et al., 1982a).

Kinetics of Autodigestion. Figure 2 gives the time course of repressor autodigestion at two pH values. It is evident that at both pH values the reaction is first-order. Values of the apparent rate constant k_{app}, used in the following analyses, were calculated routinely from such plots.

From Figure 2, k_{app} at pH 10.70 and 37 °C was 1.8×10^{-3} s^{-1} for LexA and 4.6×10^{-5} s^{-1} for λ repressor. At pH 8.65 the corresponding k_{app} values are 1.6×10^{-4} and 3.1×10^{-6} s^{-1}. At both pH values, autodigestion of LexA protein was 40–50 times faster than that of λ repressor, a difference similar to that seen in vivo and in the RecA-dependent in vitro reaction [cf. Little and Mount (1982) and Little (1983)].

Figure 2 shows that change in pH affects the rate of the autodigestion reaction but not the strict adherence to first-order kinetics. Departure from first-order kinetics was observed for severe experimental conditions, such as pH above 12 or temperatures greater than 52 °C. Data from such experiments may reflect a time-dependent denaturation step and were not included in the following analyses.

Dependence of the Rate of Autodigestion on Repressor Concentration. Intramolecular reactions are expected to follow first-order kinetics and to have a concentration-independent rate constant. However, dimerization of the reactant can complicate interpretation of rate data. Specifically, if autodigestion of a dimerizing protein is measured at a concentration above the dimer dissociation constant, then rate data would not distinguish an intramolecular process from an intermolecular process in which one subunit of a dimer attacks and cleaves the other subunit.

λ Repressor undergoes dimerization and binds to operator DNA as a dimer (Chadwick et al., 1970; Pirrotta et al., 1970; Sauer, 1979). At pH 8.0, 0 °C, and 200 mM KCl, the dissociation constant K_{dimer} of the λ repressor dimer is about 2×10^{-8} M, as determined by equilibrium measurements with gel filtration chromatography (Sauer, 1979). A similar value was obtained at pH 7.0, 22 °C and 50 mM KCl. Sedimentation studies at repressor concentrations of 10^{-10}, 10^{-7}, and 10^{-5} M yielded svedberg values near those expected for monomers, dimers, and tetramers, respectively (Chadwick et al., 1970; Pirrotta et al., 1970). By analogy with λ repressor, LexA protein is also thought to dimerize and to bind to operator DNA as a dimer. Equilibrium sedimentation analysis has given a value of 4.8×10^{-5} M for the LexA dimer dissociation constant (Schnarr et al., 1985). Since the intracellular concentration of LexA is probably much lower than this estimate of K_{dimer}, the observation suggests that the in vivo concentration of LexA dimers varies as the square of the monomer concentration, making the extent of repression highly sensitive to this value. For the present purposes, the above data indicate that we can achieve concentrations, below 10^{-8} M, at which both LexA and λ repressors should be predominantly monomeric.

Two lines of evidence have suggested that only monomers of λ repressor are good substrates for the RecA-dependent cleavage reaction. First, the rate of cleavage as a function of substrate concentration parallels the concentration of monomers rather than that of total repressor (Phizicky & Roberts, 1980). Second, a mutant form of the protein, Ind^s, dimerizes less efficiently than wild-type repressor (Cohen et al., 1981) and is cleaved faster at high concentrations (Cohen et al., 1981; Crowl et al., 1981). Estimation of K_{dimer} for the Ind^s mutant repressor from the rate of its cleavage relative to wild-type repressor in RecA-mediated reactions yielded a value of about 5×10^{-7} M (D. Burbee and J. Roberts, personal communication). Comparable data concerning the effect of increasing LexA concentration on the rate of RecA-dependent cleavage are not available.

To determine the effect of repressor concentration on the rate of autodigestion, k_{app} values for LexA protein, for λ repressor, and for the Ind^s mutant of λ repressor were measured over a 10^4-fold range of protein concentration, from 10^{-9} to 10^{-5} M, at 37 °C and various pH values. These experiments gave a simple result for LexA but showed more complex behavior for λ repressor.

For LexA repressor, k_{app} was found to be independent of protein concentration at pH 7.34 and 8.65 (data not shown). This indicates either that both monomers and dimers of LexA protein react or that dimers of LexA protein do not exist under the experimental conditions. We conclude that autodigestion of LexA protein is an intramolecular reaction involving isolated monomers and perhaps also monomers that are part of a dimer.

In contrast, the wild type and the Ind^s mutant of λ repressor were cleaved with a concentration-independent rate constant only at high pH (pH 10.47). At pH 8.65, increase in the concentration of λ repressor from 10^{-9} to 10^{-5} M resulted in a progressive decrease in k_{app} (Figure 3). Since this concentration range brackets that over which λ repressor dimerizes (Sauer, 1979; see above), and since the dimerization-deficient Ind^s mutant was affected only at much higher concentrations, the results suggests that only monomers undergo autodigestion efficiently and that high pH destabilizes the dimer and thereby abolishes the concentration dependence (see also Discussion).

The simplest mechanism to account for only monomers undergoing autodigestion is

$$R_2 \underset{}{\overset{K_{dimer}}{\rightleftharpoons}} 2R \qquad (1)$$

$$R \xrightarrow{k_1} P$$

$$d[P]/dt = k_1[R] = k_{app}[R_t]$$

$$k_{app} = ([R]/[R_t])k_1$$

$$[R_t] = [R] + 2[R_2]$$

$$K_{dimer} = [R]^2/[R_2]$$

where $[R_2]$, $[R]$, and $[P]$ are respectively the molar concentrations of dimer, monomer, and cleaved products. The total molar concentration of repressor $[R_t]$ is calculated on a monomer basis. The rate constant for autodigestion of free monomer is k_1. It follows from eq 1 that the rate constant ratio k_{app}/k_1 is equal to the fraction of repressor as free monomer $[R]/[R_t]$.

Figure 3 shows the dependence on repressor concentration of $k_{app}/k_1 = [R]/[R_t]$ for the wild type and the Ind^s mutant of λ repressor at pH 8.65 and 10.47. The values of the rate constant k_1 are assumed to be the same as the k_{app} values obtained at 10^{-9} M total repressor concentration, where the repressor should be largely dissociated and $[R]$ approximately equal to $[R_t]$. At pH 8.65, k_1 is 4.8×10^{-6} s^{-1} for the wild type and 4.5×10^{-6} s^{-1} for the mutant repressor. The solid curves of Figure 3 were calculated from eq 1 with values for K_{dimer} of 3.2×10^{-7} and 3.2×10^{-5} M for the wild type and the Ind^s mutant, respectively. Both dissociation constants are higher than previously observed (see above), which may reflect the higher pH and temperature of these studies. The rate data for the two repressor proteins are in agreement with the theoretical curves, suggesting that the dimers of λ repressor react slowly or not at all, as observed in RecA-dependent reactions. This conclusion is buttressed by the observation that the Ind^s and wild-type proteins exhibit approximately equal values of k_1 (Figure 3), whether compared at pH 10.47 (4.4×10^{-5} s^{-1} for wild type and 4.6×10^{-5} s^{-1} for Ind^s repressor) or at pH 8.65 (see above), indicating that the mutation in Ind^s only affects dimerization and is inconsequential for intramolecular cleavage.

The Active Site for Autodigestion within the Carboxy-Terminal 60% of λ Repressor. A tryptic fragment of LexA protein extending from residue Leu-68 to the end of the polypeptide chain at residue Leu-202 retains the scissile Ala-84–Gly-85 bond and undergoes autodigestion and RecA-mediated cleavage (Little, 1984). An analogous papain-generated fragment of λ repressor (fragment a) comprising residues 93–236 and retaining the Ala-111–Gly-112 cleavage sequence (Pabo et al., 1979) is cleaved in standard RecA-mediated reactions to yield the usual carboxy-terminal fragment and a 19-residue peptide (Sauer et al., 1982a).

A partially purified preparation of fragment a (see Materials and Methods) was incubated at pH 10.70 and 37 °C. It underwent autodigestion, presumably at the same Ala–Gly bond, as judged by the production of a fragment that comigrated with the carboxy-terminal fragment produced by autodigestion or RecA-mediated cleavage of intact repressor (data not shown). Similar observations have been made with a protein fragment of the same size but encoded by a λ repressor gene truncated from the 5′ end (R. Sauer, personal communication). Rate measurements, with an uncertainty of about 50%, indicate that the kinetics of autodigestion of fragment a were the same as for intact repressor. These observations indicate that the active site for autodigestion of

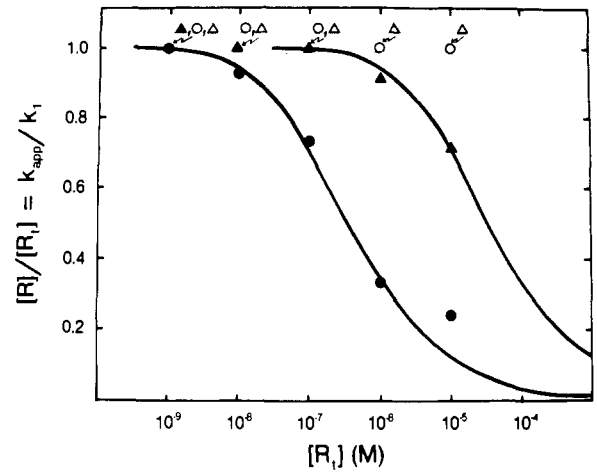

FIGURE 3: Dependence on total repressor concentration $[R_t]$ of the apparent rate constant for the autodigestion of wild type (round symbols) and Ind^s mutant (triangular symbols) of λ repressor at pH 8.65 (filled symbols) and 10.47 (open symbols). The measured values of k_{app} are plotted according to eq 1. Values of k_1 for pH 8.65 and 10.47 are given in the text. Autodigestion reactions were carried out at 37 °C with a constant amount of radiolabeled repressor and with unlabeled repressor added to obtain the desired total repressor concentration.

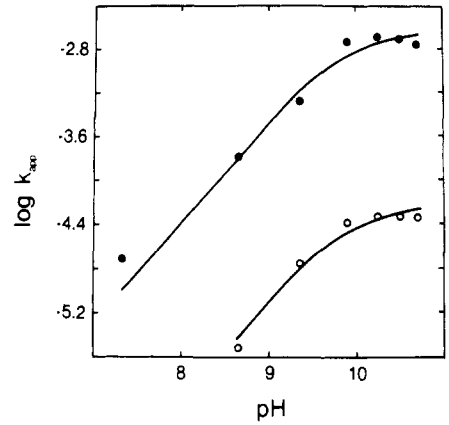

FIGURE 4: Dependence of log k_{app} on pH for the autodigestion reaction of LexA (filled symbols) and λ repressor (open symbols) at 37 °C. Total repressor concentrations are as in Figure 2. A least-squares fit of the data to the model of eq 2 gave estimates for the pK and k_{max} of, respectively, 9.82 and 2.5×10^{-3} s^{-1} for LexA and 9.84 and 6.1×10^{-5} s^{-1} for λ repressor. The curves are drawn for these parameter values. The uncertainties are 0.11 in pK and 14% in k_{max}. The root-mean-square error of the fit is 0.089 for the LexA and 0.080 for the λ repressor data.

λ repressor is in the hinge and/or the carboxy-terminal domain, as for the LexA repressor.

Dependence of the Rate of Autodigestion on pH. The pH dependence of a reaction provides information necessary for distinguishing possible mechanisms. Figure 4 gives the pH–rate profile for the intramolecular cleavage of LexA and λ repressors at 37 °C. The simplest model that can account for the data is based upon the deprotonated form of a protein ionizable group being required for activity. Equation 2 ac-

$$RH^+ \underset{}{\overset{K_d}{\rightleftharpoons}} R + H^+ \qquad (2)$$

$$R \xrightarrow{k_{max}} P$$

$$d[P]/dt = k_{max}[R] = k_{app}[R_t]$$

$$[R_t] = [RH^+] + [R]$$

$$k_{app} = k_{max}/(1 + [H^+]/K_d)$$

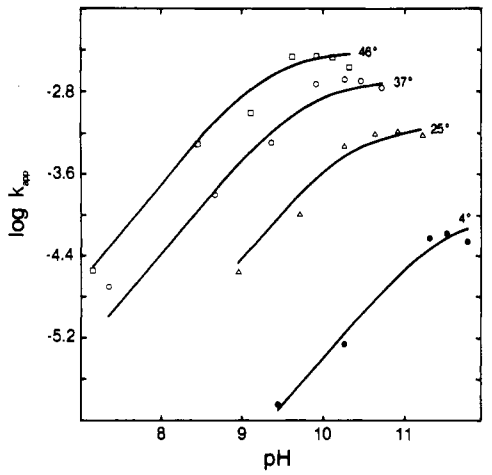

FIGURE 5: Dependence of log k_{app} on pH for autodigestion of LexA protein at various temperatures. Reactions were performed at 4 (filled circles), 25 (open triangles), 37 (open circles), and 46 ± 1 °C (open squares) as described under Materials and Methods, with a total repressor concentration of 6×10^{-6} M. The pH of each reaction mixture was measured at the reaction temperature. The curves were calculated for parameters from a least-squares fit of the model described in the text. Estimates for the pK and k_{max} are respectively 11.41 and 1.1×10^{-4} s^{-1} at 4 °C, 10.30 and 7.8×10^{-4} s^{-1} at 25 °C, 9.73 and 2.1×10^{-3} s^{-1} at 37 °C, and 9.33 and 4.3×10^{-3} s^{-1} at 46 °C. The uncertainties are 0.06 in pK and 9% in k_{max}. The root-mean-square error for the fit was 0.082.

curately describes the pH dependence of the autodigestion process. The solid curves of Figure 4 were drawn as a least-squares fit of the LexA and λ repressor data to eq 2. The LexA and λ repressors exhibit identical apparent pK values, 9.82 ± 0.12 and 9.84 ± 0.11, respectively. This observation is consistent with the mechanism of cleavage being the same for the two repressors.

Dependence of the Rate of Autodigestion on Temperature. Figure 5 shows the rate of autodigestion of LexA protein over the range of pH 7.15–11.77, at 4, 25, 37, and 46 °C. Extension of eq 2 to take into account temperature dependence of the rate and equilibrium constants gives the relationships

$$k_{app} = k^{T}_{max}/(1 + [H^+]/K^{T}_{d}) \quad (3)$$

$$k^{T}_{max} = k^{298}_{max} \exp\{(\Delta H^{\ddagger}/R)(1/298 - 1/T)\}$$

$$K^{T}_{d} = K^{298}_{d} \exp\{(\Delta H_d/R)(1/298 - 1/T)\}$$

where k_{app}, k_{max}, and K_d are as for eq 2, except that they refer to the indicated temperature (experimental or 25 °C as the reference), and $\Delta H^{\ddagger}$ and ΔH_d are the enthalpies of activation and proton dissociation. The model of eq 2 and 3 was fit to the data of Figure 5, giving the parameter values of Table I and the legend of Figure 5. The curves of Figure 5 are drawn for this set of parameters.

Dependence of the Rate of Autodigestion on Buffer Concentration. A solvent component, such as hydroxide ion or other base species, might participate as a general catalyst or as a nucleophile in one or more steps of the pathway for peptide bond hydrolysis. This possibility was examined by two sets of experiments. First, the rate constants for autodigestion of LexA and λ repressors were determined at high concentrations of buffer. Ionic strength was maintained constant at 1.0 with sodium chloride, because the autodigestion reaction displayed approximately 35% reduction in rate in the presence of 1 M NaCl (data not shown). The buffer pH was set at values chosen such that at one pH the ratio of the basic to acidic species was 7/3 and at the other pH, 3/7. For λ repressor, k_{app} determinations were made for increasing concentrations

Table I: Thermodynamic Parameters for Proton Dissociation and Bond Rearrangement Steps of LexA Protein Autodigestion at 25 °C

process	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS [cal/(K·mol)]
ionization	14.1 ± 0.1	19.9 ± 1.5	19.6
activation	21.7 ± 0.1	15.3 ± 1.0	−21.4

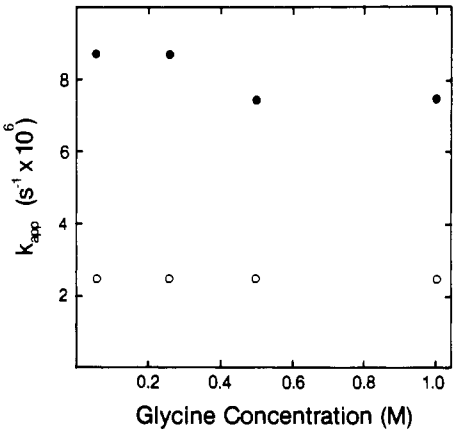

FIGURE 6: Effect of increasing glycine buffer concentration on λ repressor autodigestion at pH 9.14 (open symbols) and 9.88 (filled symbols) at 37 °C. Reactions were performed at 37 °C at a repressor concentration of 1×10^{-9} M.

of glycine at pH 9.14 and 9.88 at 37 °C. For LexA protein, which reacts faster than λ repressor (Figure 2), the measurements were performed with imidazole at pH 6.39 and 7.13, at 37 °C. The data of Figure 6 show that k_{app} for autodigestion of λ repressor is not affected by the highest concentration of buffer at both pH. Similar results were obtained for LexA (data not shown).

In the second test, hydroxylamine was used at concentrations up to 0.5 M, at 0.5 ionic strength, and at pH 7 or 8. This nucleophile also did not affect the rate of autodigestion.

Dependence of the Rate of Autodigestion on Metal Ion. As a test for the involvement of metal ions, the autodigestion reaction was studied under metal-free conditions. LexA protein was denatured by dialysis against a buffer containing 6 M guanidine hydrochloride and the metal-ion chelating agents EDTA, EGTA, and Chelex and then renatured by exhaustive dialysis against the same buffer without guanidine hydrochloride. The preparation was then autodigested with and without added divalent cations at pH 10.20 and 37 °C. In the absence of added divalent cations, the reaction proceeded with first-order kinetics and at a rate almost as rapid as with untreated protein. The reaction rate was stimulated about 1.5-fold upon the addition of 10 mM Group IIA (group 2)[2] ions Mg^{2+}, Ca^{2+}, Sr^{2+}, or Ba^{2+} but not the transition metals Co^{2+}, Cu^{2+}, or Zn^{2+}. The rate with divalent metal ion is equal to that determined for untreated LexA repressor. The 1.5-fold stimulatory effect is smaller than the 2–4-fold effect previously reported (Little, 1984). These observations indicate that the chemistry of the intramolecular cleavage reaction does not require participation of a divalent cation.

In a less-extensive set of experiments, we studied autodigestion of λ repressor, using a protein preparation that had not

[2] In this paper the periodic group notation in parentheses is in accord with recent actions by IUPAC and ACS nomenclature committees. A and B notation is eliminated because of wide confusion. Groups IA and IIA become groups 1 and 2. The d-transition elements comprise groups 3 through 12, and the p-block elements comprise groups 13 through 18. (Note that the former Roman number designation is preserved in the last digit of the numbering: e.g., III → 3 and 13.)

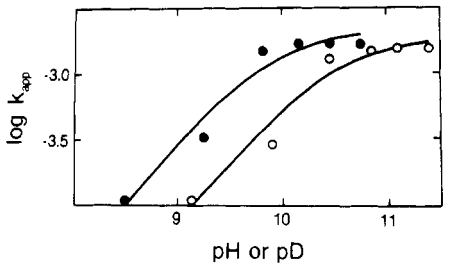

FIGURE 7: Solvent deuterium isotope effect on log k_{app} for the autodigestion reaction of LexA protein at 37 °C. Reactions were carried out as described under Materials and Methods with H_2O (filled symbols) or D_2O (open symbols) and a total LexA concentration of 6×10^{-6} M. A least-squares fit of the data to the model of eq 2 gave estimates for the pK and k_{max} of, respectively, 9.83 and 2.2×10^{-3} s^{-1} for H_2O and 10.44 and 2.0×10^{-3} s^{-1} for D_2O. The ratio of reaction rates k_H/k_D, in the plateau region of the pH–rate profile, was 1.1. The uncertainties are 0.15 in pK, 17% in k_{max}, and 0.3 in the rate ratio. The root-mean-square errors of the fits were 0.115 and 0.111, respectively, for the H_2O and D_2O data.

been treated to remove metal ions as described above. The rate of autodigestion was followed at pH 9.40 and 37 °C, with and without 10 mM $CaCl_2$, at repressor concentrations below those affecting k_{app}. In these measurements, $CaCl_2$ gave about a 6-fold stimulation of k_{app} (data not shown), a larger effect than observed above with LexA repressor.

Solvent Deuterium Isotope Effect. Use of a heavy isotope of hydrogen provides information concerning proton transfer in the rate-determining step. Figure 7 shows the dependence of k_{app} on pH or pD for LexA autodigestion at 37 °C. The experimental conditions were identical for the two sets of data, except for the substitution of D_2O for H_2O. The curves of Figure 7 are drawn for parameters estimated by fitting the H_2O and D_2O data to eq 2. The data for D_2O are displaced to higher pH by about 0.6 unit, resulting in an apparent pK value of 10.44 ± 0.15 as compared to the 9.84 ± 0.15 value for H_2O. A shift of this magnitude is expected for transfer of an ionizable group from H_2O to D_2O (Bunton & Shiner, 1961) and is consistent with the model described above, in which deprotonation of an active site group is required for activity.

The ratio k_H/k_D in the plateau region of the pH/pD–rate profile does not differ significantly from unity (Figure 7).

Discussion

Autodigestion of LexA Repressor. Two lines of evidence indicate that autodigestion of LexA repressor is an intramolecular reaction. First, cleavage proceeds with first-order kinetics (Figure 2); second, its rate constant is independent of protein concentration over a wide range. Each of these findings, by itself, would be compatible with intermolecular catalysis. First-order kinetics might be obtained below the K_m of an intermolecular reaction, if the products catalyzed cleavage; apparent concentration independence might be observed above the K_m. Taken together, however, the two lines of evidence can only be reconciled with an intramolecular reaction.

Autodigestion of λ Repressor. The autodigestion reaction of λ repressor also proceeds with first-order kinetics. The rate constant is independent of total repressor concentration at pH 10.47, but at pH 8.65 it decreases with increasing repressor concentration (Figure 3). The profile at pH 8.65 closely follows the model of eq 1, for which repressor monomers are reactive and dimers are unreactive, with K_{dimer} 3×10^{-7} M.

Other evidence supports the conclusion that the concentration dependence at pH 8.65 results from dimerization of the protein. First, equilibrium measurements have shown that λ repressor dimerizes, with K_{dimer} 2×10^{-8} M under conditions different from those of this study (Sauer, 1979). Second, the Ind^s mutant protein, known to dimerize less efficiently than wild-type repressor (Cohen et al., 1981), autodigests at the same rate as wild-type protein at low concentrations, and it shows concentration dependence of the rate constant at a 100-fold higher concentration than the wild type (Figure 3). This pattern suggests that at low concentrations both proteins are monomers and show the same intrinsic rate of autodigestion and that as the repressor dimerizes, its rate of cleavage drops.

Another interpretation of the concentration dependence is plausible. The value of K_{dimer} inferred above is some 15-fold larger than that determined by Sauer (1979). This discrepancy suggests either that dimerization is the important concentration step, and that the dimerization constant is different under the present conditions than those of Sauer, or, alternatively, that the observed concentration dependence results not from dimerization but from some other form of aggregation involving dimers. This second hypothesis accounts for the difference between wild-type and Ind^s repressors by assuming that the inactive oligomer would be formed from dimers. Since we have not measured the dimerization constant under our conditions, we cannot distinguish between these two possibilities. We did observe in preliminary experiments at pH 9.4 and 37 °C that the concentration-dependent decrease in k_{app} took place in the presence but not the absence of 10 mM $CaCl_2$, indicating that $CaCl_2$ alters the concentration-dependent process.

RecA-Dependent Cleavage and Autodigestion Share the Same Reaction Pathway. The present results lend further support to the hypothesis (Little, 1984) that RecA is an activator of autodigestion rather than a specific protease. First, the two types of cleavage reaction result in the same chemical rearrangement, hydrolysis of the conserved Ala–Gly bond near the center of the protein; second, the ratio of the rates of cleavage of LexA and λ repressors (Figure 2) is similar under autodigestion conditions and in the presence of RecA; third, for both repressors the RecA-dependent and autodigestion reactions are observed for proteolytic fragments containing the carboxy-terminal domain and the hinge region including the Ala–Gly cleavage site.

A possible fourth point of similarity between the RecA-dependent and autodigestion reactions with λ repressor as substrate is that both types of reactions are affected by repressor concentration. However, we cannot yet be certain that the concentration dependence operates by the same mechanism in the two cases.

Comparison of LexA and λ Repressors. LexA protein autodigests 40–50 times faster than does λ repressor (Figure 2). This difference, which is similar to the disparity in the in vivo and the RecA-dependent in vitro rates, may be of biological significance, in that it provides the λ prophage with a threshold of DNA damage tolerance below which the cell repairs the damage and allows the lysogenic state of the virus to continue [see also Shea and Ackers (1985)]. This is in contrast to the need for the cellular SOS system under LexA control to respond rapidly to low levels of DNA damage. Our data suggest that the rate difference is an intrinsic property of the repressors, rather than primarily resulting from significant differences in their interaction with activated RecA protein.

In addition, the present data lend further support to the previous conclusion that LexA and λ repressors have parallel structural and functional organization [cf. Little and Mount

(1982) and Little and Hill (1985)]. In this work we have shown that the autodigestion reactions of both LexA and λ repressors are intramolecular, that cleavage activity is contained within the carboxy-terminal 60% of λ repressor, as with LexA (Little, 1984), and that the two repressors display the same pK for the autodigestion reaction, suggesting that the mechanism of this reaction is the same for both proteins.

Kinetic Parameters and Pathway. The pH–rate profiles of LexA and λ repressors are consistent with the mechanism of eq 2 and 3, in which an ionizable group on the protein must be deprotonated for activity. The mechanism gives a satisfactory description, with the use of only four variable parameters, of the rate data over the full range of pH and temperature. There is no need to invoke a mechanism of greater complexity, although of course more complex mechanisms cannot be excluded.

Barring a change in the rate-determining step at pH near 10, for any plausible mechanism the pK inferred from the kinetic analysis would be equal to a thermodynamic pK, i.e., one measured by titration. Thus, we can conclude that the unprotonated species of a protein group of apparent pK 10.3 at 25 °C (Figure 5) participates in the hydrolysis reaction (see also the next section).

The 20 kcal/mol heat of dissociation (Table I) of this group is about twice the normal value for ionization of basic side-chain groups. Possibly this large value includes contributions from other processes, such as hydrogen-bond rearrangements or small conformational changes.

The high-pH limiting rate (k_{max}) for hydrolysis of the Ala–Gly bond of LexA represor (7.8×10^{-4} s^{-1} at 25 °C, Figure 5) may be compared with those for hydrolysis of typical amides. It is about 500 times smaller than the rate of amide hydrolysis catalyzed by chymotrypsin (Green & Neurath, 1954; Bruice & Benkovic, 1966) and 25 000 times larger than the rate for hydroxide ion catalyzed amide hydrolysis (Bender et al., 1964).

The heats of ionization and activation, 20 and 15 kcal/mol, respectively, are small compared to heats of protein denaturation, which fall in the range of 50–100 kcal/mol (Lapanje, 1978; Hawkes et al., 1984). This observation suggests that there is not significant unfolding of the protein associated with the early steps of the autodigestion reaction. This conclusion is also consistent with measurements of the steady-state fluorescence of LexA and λ repressors, which for both repressors does not change between pH 9.70 and pH 11.20 at 25 °C nor during the course of autodigestion at pH 10.63 and 25 °C for LexA protein (S. Slilaty, unpublished data).

Solvent species did not compete as nucleophiles or general acid–base catalysts in the autodigestion reaction. These observations support the model described above, in which the apparent pK near 10 reflects ionization of a protein group required for activity, and argue against alternative mechanisms in which hydroxide ion participates as a nucleophile.

The absence of a significant deuterium solvent isotope effect is most consistent with proton transfer not occurring in the rate-determining step.

Mechanism of Repressor Autodigestion. The mechanistic data presented above and several preliminary experiments described below suggest, in the light of repressor homology and the precedents of proteolytic reactions, a mechanism for the autodigestion reaction.

With regard to the identity of the ionizable group of pK near 10, LexA contains no cysteine residues (Horii et al., 1981b; Markham et al., 1981), and there is no histidine or tyrosine homology between LexA and phage repressors (Sauer et al., 1982b). Lys-156 of LexA is the only conserved basic residue and thus is identified as the likely candidate for an active site group of pK near 10. By use of site-directed mutagenesis, Lys-156 has been changed to Ala (S. Slilaty, unpublished data). Preliminary measurements indicate that this alteration abolishes activity under both autodigestion and RecA-dependent cleavage conditions, yet the protein appears to retain full repressor activity in vivo. Thus, the unprotonated form of the Lys-156 side chain may be required, perhaps as an activator of a nucleophile such as a water molecule or a protein serine hydroxyl group, which in turn attacks and effects cleavage of the scissile peptide bond.

Self-Processing Macromolecules. A number of recent reports have shown the existence of macromolecules with a behavior analogous to that of the repressors described here. These may be denoted collectively as "self-processing macromolecules". Their common features are, first, that they exhibit intramolecular covalent rearrangement, second, that this rearrangement is facilitated by the folding of the macromolecule, and finally, that the rearrangement alters the function of the macromolecule. The last property resembles zymogen activation, except that most zymogen reactions are intermolecular. Self-processing is distinguished from true enzyme-mediated catalysis (see also below) in that the molecule does not emerge from the reaction unchanged, but one can think of the chemical events of self-processing in much the same way as one thinks of enzyme catalysis.

Examples of self-processing macromolecules have been observed in prokaryotic and eukaryotic systems. The proenzyme of histidine decarboxylase of *Lactobacillus* undergoes activation via an intramolecular process in which the bond between Ser-81 and Ser-82 is broken, and Ser-82 is converted to a pyruvoyl unit that becomes part of the active site of the mature enzyme (Recsei et al., 1983). One of the early steps in the activation pathway of the eukaryotic proenzyme pepsinogen is an intramolecular cleavage reaction (Marciniszyn et al., 1976; Auer & Glick, 1984). The initial step in the processing reaction of the poliovirus proteins appears to be the self-excision of the processing protease from the precursor polyprotein chain (Hanecak et al., 1984). At least one intramolecular cleavage event has been observed in the production of functional encephalomyocarditis viral proteins from the large precursor polyprotein (Palmenberg & Rueckert, 1982). Recent evidence suggests that the *Drosophila* heat-shock protein hsp70 can autodigest to give a variety of products; this reaction may serve to make the protein unstable in vivo, thereby providing a mechanism for the cell to return to the normal state (Mitchell et al., 1985).

The best characterized self-processing reaction is the excision of intervening sequences from *Tetrahymena* rRNA transcripts (Kurger et al., 1982; Zaug et al., 1983, 1985). In this case (and perhaps with the poliovirus polyprotein), the active site can be considered a true catalyst, since it can carry out analogous intermolecular reactions when provided with appropriate substrates (Zaug & Cech, 1986).

In contrast to the self-splicing RNA molecules, introns of most eukaryotic mRNA's are excised by a complex enzymatic machinery. Sharp (1985) has speculated that, early in evolution, this type of intron was also self-splicing and that only later did trans-acting enzymes evolve that carried out the reaction more efficiently. At this point, the self-splicing properties of the introns were no longer needed and were lost during the course of evolution.

We speculate that a parallel evolutionary process has taken place in the case of the repressors studied here. According

to this hypothesis, the self-processing archetypal repressor evolved first; only later did activated RecA evolve as an effector of the reaction. For this archetypal repressor to regulate gene expression in response to change in external conditions, its rate of autodigestion must have been controlled by one or more signal molecules. When RecA evolved to sense the environment, there was no longer a need for the repressor to do so. The response property of the repressor, but not the property of autodigestion, was presumably lost during evolution, since activated RecA is required for derepression of the current SOS system (Little & Mount, 1982; Walker, 1984). Alternatively, the original signal might have been one that can no longer reach the repressor; for example, if the signal had been high pH, the cell later would have evolved to better control its internal pH. Corollaries of this proposal are that λ and other prophage repressors diverged from the ancestral LexA after the protein became responsive to activated RecA and that the intrinsic rates of cleavage (see above) evolved to optimum values in each case.

Apparently, self-processing is not an uncommon approach to the solution of a variety of biological regulatory problems. Because recognition of the existence of self-processing macromolecules has been recent, one can expect the number of known examples of this class to increase substantially.

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DNA Cleavage Specificity of a Group of Cationic Metalloporphyrins†

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ABSTRACT: The ability of a group of water-soluble metalloporphyrins to cleave DNA has been investigated. Incubation of Mn^{3+}, Fe^{3+}, or Co^{3+} complexes of *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphine (H_2T4MPyP) with DNA in the presence of ascorbate, superoxide ion, or iodosobenzene results in DNA breakage. Comparisons between the rates of porphyrin autodestruction with the rates of strand scission of covalently closed circular PM2 DNA indicate that the porphyrins remain intact during the cleavage process. Analysis of the porphyrin-mediated strand scissions on a 139-base-pair restriction fragment of pBR322 DNA using gel electrophoresis/autoradiography/microdensitometry reveals that the minimum porphyrin cleavage site is $(A{\cdot}T)_3$. The cleavage pattern within a given site was found to be asymmetric, indicating that porphyrin binding and the strand scission process are highly directional in nature. In addition to an analysis of the mechanism of porphyrin-mediated strand breakage in terms of the DNA cleavage mechanism of methidium–propyl–iron–EDTA and Fe–bleomycin, the potential of the cationic metalloporphyrins as footprinting probes and as new "reporter ligands" for DNA is presented and discussed.

The interaction of metal ions with DNA is of considerable current interest (Eichhorn, 1973; Eichhorn & Marzilli, 1982; Barton, 1985). In addition to simple aquated metal salts, several complexes containing a variety of different coordinated ligands are known to interact with DNA. For example, the exchange-inert metal complex $[Co(NH_3)_6]^{3+}$ has been shown to be an effective inducing agent for stabilizing left-hand helical DNA, termed Z-DNA, (Behe & Felsenfeld, 1981). X-ray crystallographic studies have indicated that the compound exerts its effects through electrostatic and hydrogen-bonding interactions to groups located in the major groove of DNA (Gessner et al., 1985). In addition to $[Co(NH_3)_6]^{3+}$, compounds of Cu^{2+}, Zn^{2+}, and Co^{3+} having multidentate amine type ligands have been found useful in studies of DNA conformation (Barton, 1985; Fazakerly, 1984; Woisard, et al. 1985). The exchange-inert complex ion tris(4,7-diphenylphenanthroline)cobalt(III) is especially noteworthy in this regard since the two enantiomers of the cation bind to DNA with different apparent affinities. Furthermore, the compound can be photoactivated to produce strand scission, thus clearly identifying the regions of Z-conformation in DNA (Barton & Raphael, 1985).

Nuclease activity also can be elicited from the complex ion bis(*o*-phenanthroline)copper(I), (Spassky & Sigman, 1985). Recent studies of this cation with wild-type, Ps, and L8-UV-5 *lac* promoters have suggested that the compound is sensitive to sequence-dependent structural changes in DNA.

One of the most versatile metal-containing agents for studying drug–DNA interactions is the synthetic DNA cleaving agent methidium–propyl–iron–EDTA, MPE (Van Dyke et al., 1982). This compound, which contains an iron–EDTA moiety tethered to a methidium group, has been used in footprinting studies to uncover the binding sequence specificities of a number of anticancer drugs and other ligands capable of equilibrium binding to DNA. The success of the compound as a footprinting probe is related to the fact that it can bind to DNA via intercalation and at the same time produce a flux of DNA-damaging radicals through reductive activation of the tethered iron–EDTA moiety. In addition to attachment to methidium, the iron–EDTA group also has been tethered to a number of *N*-methylpyrrole oligopeptides. The strand scission sites produced by these modified oligopeptides on defined-sequence DNA have provided valuable information on the manner in which naturally occurring groove-binding peptides such as netropsin and distamycin are able to "read"

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